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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/448,633	11/24/1999	AI-PING WEI	53091USA8B	5266

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EXAMINER	
MORAN, MARJORIE A	

ART UNIT	PAPER NUMBER
1631	19

DATE MAILED: 02/10/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Application No.	Applicant(s)	
09/448,633	WEI ET AL.	
Examiner	**Art Unit**	
Marjorie A. Moran	1631	

Office Action Summary

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b)

Status

1)☒ Responsive to communication(s) filed on *20 November 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2 and 4-23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2 and 4-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☒ Interview Summary (PTO-413) Paper No(s). *14* .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/20/02 has been entered.

Claims 1-2 and 4-21 are pending. All rejections and objections not repeated below are hereby withdrawn.

Claim Objections

Claims 2 and 14 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Parent claims 1 and 12 recite a compound and method of use of the compound wherein the compound comprises two identical dye groups. Dependent claims 2 and 14 limit the dye groups to be identical, and therefore fail to further limit their respective parent claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-2, 4-8 and 10-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over GARMAN *et al.* (GB 2278356) as supported by ROHATGI *et al.* (J. Phys. Chem. (6/1966) vol. 70 (6), pages 1695-1701) and WEI *et al.* (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506), and in view of KOMORIYA et al. (US 5,714,342).

Claim 12 recites a protease substrate comprising two identical fluorescence dye groups bound to a flexible peptide wherein the dye groups are close enough to self-quench through intramolecular dimerization or dye-stacking, and which, when separated, fluoresce at a 10-fold or more increase in intensity over quenched dye groups, and wherein the emission wavelength of the dye groups is 570 nm or greater. Claim 1 recites a biological assay method comprising providing an enzyme substrate similar to that of claim 12 wherein the substrate is further limited to comprise one or more enzymatically cleavable bonds; and the method also comprises a step of cleaving the one or more cleavable bonds to thereby produce an increase in fluorescence intensity of at least 10-fold over that of the quenched dye groups. Claims 2 and 14 further limit

the dye groups to be identical. Claim 15 further limits the dye groups of the substrate of claim 12 to comprise a fluorescence donor and acceptor. Claims 4 and 13 limit the distance between the dye groups to be less than 100 ´. Claims 6 and 16 further limit the dye groups to be planar. Claims 7-8 and 17-18 further limit the dye groups to be fluorescein, rhodamine, cyanine, tetramethylrhodamine (TMR), X-Rhodamine, Rhodamine B, or TEXAS RED. Claim 10 further limits the method to one wherein enzymatic cleavage is performed by an aspartic, metallo-thio, serine, retroviral, or trypsin protease. Claim 11 further limits the method to one wherein fluorescence intensity is increased at least 10-fold compared to an intensity increase in conventional assay kits comprising a protease substrate.

GARMAN teaches a protease substrate (p. 13) and assay method using the substrate wherein the substrate is cleaved by an enzyme and an increase in fluorescence is observed (pp. 14-15), wherein the substrate comprises a flexible peptide and two fluorescence groups which are fluorescein and tetramethylrhodamine (substrate D on pages 13-14). GARMAN also teaches substrates comprising two groups with the same fluorophore (substrate 3 on page 11). ROHATGI provides support that both fluorescein and tetramethylrhodamine are capable of dye-stacking (p. 1696 and 1699) and WEI provides support that fluorescein and tetramethylrhodamine attached to a peptide can interact to "essentially" self-quench the fluorescence groups (p. 1503, Figure 3A). GARMAN's substrate may comprise Peptide II (p. 13), which is 14 amino acids in length. As each amino acid is 3.8 Å, a peptide of 14 amino acids is about 53.2 Å, therefore if the C-terminus and N-terminus are labeled with the dye groups (i.e. the furthest distance possible), the dye groups will be separated by at most 53.2 Å. WEI also provides support that distances of 47-54 Å allow up to 70%

quenching of fluorescence (pp. 1503-1504). The Staphylococcal V8 protease of GARMAN is an aspartic protease. GARMAN, as supported by ROHATGI and WEI, does not teach "at least" a 10-fold increase in fluorescence intensity in his method compared to "conventional assay kits" using a protease substrate; nor does GARMAN, as supported by ROHATGI and WEI teach a substrate wherein the dye groups are identical, have emission wavelengths of 570 nm or more, and give at least a 10-fold increase in fluorescent intensity upon separation of the dye groups.

KOMORIYA teaches a protease substrate comprising an enzyme cleavage site and two fluorescent dyes, wherein said dyes undergo quenching due to dye stacking, or dimerization (col. 9, lines 5, 25-26 and col. 31, line 45-col. 33, line 15). He also teaches a variety of enzymes for use with said substrate (col. 12-15, Table 2), donor and acceptor fluorophores of planar configuration, preferably tetramethylrhodamines and rhodamine X acetamide (col. 15, line 43-col. 16, line 37). KOMORIYA also teaches that use of a double fluorophore substrate shows a dramatic increase in fluorescent intensity compared to the same method using another substrate (col. 30, lines 53-56). KOMORIYA specifically teaches that substrates may be doubly-labeled with the same fluorophore (col. 31, line 49-col. 33, line 15), and teaches that an advantage for use of a homo-doubly-labeled substrate is that a homo-labeled substrate requires use of only a cutoff filter whereas the hetero-labeled substrates require use of an interference filter in methods of detection (col. 34, lines 16-27). KOMORIYA further teaches that fluorogenic substrates labeled with rhodamine groups can show a "more than 10 fold" increase in fluorescence at 598 nm upon treatment with an enzyme (i.e. separation of quenched fluorescent groups), and teaches that his homo-labeled compounds show quenching similar to that of hetero-labeled compounds (col's 21-22, Table 9).

It would have been obvious to one of ordinary skill in the art at the time of invention to have labeled the substrate (Peptide II) of GARMAN, ROHATGI and WEI with identical rhodamine groups, as exemplified by KOMORIYA, such that "at least" a 10-fold increase in fluorescence at 589 nm is seen upon cleavage/separation of the quenched dye groups, as suggested by KOMORIYA's teaching that greater signal is desirable (col. 30, lines 33-37), where the motivation would have been to use any of the combination of dyes of KOMRIYA which are known to be highly quenched and thus result in high fluorescence intensity upon cleavage of an enzyme substrate, in order to optimize, or increase sensitivity, of the method, as taught by both KOMORIYA and GARMAN. As KOMORIYA teaches that singly labeled fluorophores do not show any increase in intensity upon cleavage of the labeled substrate (Figure 4), then an assay using the double-labeled substrate of KOMORIYA and GARMAN; i.e. one which results in any increase in fluorescence upon cleavage of the substrate, would necessarily show an increase in fluorescence which is at least 10-fold that of a "conventional" assay using a single-labeled substrate. One would further have been motivated to have labeled the substrate of GARMAN with any of the fluorophores taught by KOMORIYA to be satisfactory for homo-double-labeling of a protease substrate (e.g. any of those shown in Table 9 except fluorescein) in order to facilitate use of the substrate in methods of detection, as suggested by KOMORIYA's teaching that homo-double-labeled substrates supply an advantage in methods of detecting enzymes, as set forth above. One skilled in the art would reasonably have expected success in labeling the substrate of GARMAN with identical fluorophores, as taught by KOMORIYA because both KOMORIYA and GARMAN teach labeling of substrates with fluorescein groups, both teach quenching of those groups such that an increase in fluorescence is successfully detected upon cleavage of the substrate. One would reasonably have expected success in detecting a greater than 10-fold increase in fluorescence upon separation of dye

groups (cleavage of the substrate) when identical dye groups are used because KOMORIYA teaches that substrates with identical dye groups show similar quenching to hetero-labeled dye groups, and KOMORIYA teaches that a change in intensity can be increased by increasing the slit width of the detector (col. 30, lines 28-38).

Applicant argues in the response filed 11/20/02 that GARMAN and KOMORIYA can not properly be combined because GARMAN teaches quenching due to energy transfer while KOMORIYA teaches dimerization. In response, it is noted that GARMAN teaches that the same dye groups may be used on either end of a substrate in his method, as set forth above, and specifically teaches that suitable fluorophores for use in his method of detecting a protease include tetramethylrhodamine and rhodamine derivatives (p. 7). As homo-labeled examples of KOMORIYA comprise rhodamine derivatives, an specifically comprise tetramethylrhodamine (C1171 in Table 9), the examiner maintains that one skilled in the art would reasonably have expected success in homo-labeling the substrate of GARMAN with the TMR or other rhodamine derivatives of KOMORIYA. Further, as both GARMAN and KOMIRIYA teach double-labeled substrates wherein fluorescence quenched such that cleavage of the substrate results in an increase in fluorescence intensity due to "unquenching" of the dye groups, and KOMORIYA specifically teaches that homo-labeling provides results similar to that seen with a hetero-labeled compound, one skilled in the art would reasonably have expected success in homo-labeling the substrate of GARMAN to provide a compound such that cleavage of the substrate result in an increase in fluorescence. As both KOMORIYA and GARMAN teach substrates with similar properties and similar motivations for making such substrates (i.e. detection of proteases), and as one skilled in the art would reasonably have expected success in combining the substrate of GARMAN with the fluorophores of KOMIRIYA, as set forth above, the examiner maintains that one skilled in the art would have been motivated to use the fluorophores of

KOMORIYA with the substrate of GARMAN, regardless of the mechanism of quenching, for the motivations set forth in the rejection above.

Applicant further argues in the response filed 11/20/02 that one skilled in the art would not have been motivated to use dyes with an emission wavelength of 570 nm or above. In response, it is noted that the rhodamine dyes of KOMORIYA emit at 598 nm, and that both GARMAN and KMORIYA teach use of rhodamine dyes with their protease substrates.

Claims 9 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over GARMAN *et al.* (GB 2278356) as supported by ROHATGI *et al.* (J. Phys. Chem. (6/1966) vol. 70 (6), pages 1695-1701) and WEI *et al.* (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506), in view of KOMORIYA *et al.* (US 5,714,342), as applied to claims 1-2, 4-8 and 10-18 above, and further in view of HEATH, JR. *et al.* (US 5,235,039).

Applicant claims a protease substrate and biological assay using the substrate, as set forth above. Claims 9 and 19 further limit the substrate to one comprising 2-10 amino acids wherein the dye groups form a stack and the substrate comprises at least one enzyme-specific cleavable bond.

GARMAN, ROHATGI , WEI, and KOMORIYA teach and make obvious a flexible protease substrate labeled with identical fluorescent dye groups which dimerize or stack so as to self-quench, wherein the substrate comprises at least one enzyme cleavable bond, and displays the fluorescent properties claimed, as set forth above. GARMAN, ROHATGI , WEI, and KOMORIYA do not teach a substrate comprising 2-10 amino acids.

HEATH, JR. teaches an octapeptide substrate for collagenase which is fluorescently labeled (col. 10, lines 23-34).

It would have been obvious to one of ordinary skill in the art at the time of invention to have substituted the octapeptide of HEATH for Peptide II in the substrate and method of GARMAN, ROHATGI , WEI, and KOMORIYA where the motivation would have been to measure vertebrate collagenase, as taught by HEATH (col. 10, lines 35-51).

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over GARMAN *et al.* (GB 2278356) as supported by ROHATGI *et al.* (J. Phys. Chem. (6/1966) vol. 70 (6), pages 1695-1701) and WEI *et al.* (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506), in view of KOMORIYA *et al.* (US 5,714,342), as applied to claims 1-2, 4-8 and 10-18 above, and further in view of MANAFI *et al.* (Microbiol. Reviews (9/1991), vol. 55 (3), pages 335-348).

Claim 21 recites a method of detecting a microorganism using the same steps as recited in claim 1.

GARMAN, ROHATGI, WEI, and KOMORIYA make obvious a biological assay wherein activity of a Staphylococcal aspartic protease is detected, as set forth above. GARMAN does not specifically teach detection of a microorganism.

MANAFI teaches that microbial cells can be detected by detecting a change in fluorescent intensity due to cleavage of a fluorogenic substrate (p. 336). MANAFI specifically teaches that fluorescent substrates can be used to detect Staphylococcus (p. 338).

It would have been obvious to one of ordinary skill in the art at the time of invention to have detected Staphylococcus using the substrate and method of GARMAN where the motivation would have been to use cleavage of a specific fluorescent substrate to detect and differentiate Staphylococcus, as suggested by MANAFI's teaching that specific Staph. species can be detected with a fluorescent substrate.

Allowable Subject Matter

Claim 20 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The following is a statement of reasons for the indication of allowable subject matter: The prior art does not teach the structure recited in claim 20. The prior art does not teach any motivation to pick the particular amino acid sequence recited in claim 20 as the peptide portion of the claimed substrate, nor does the prior art teach the claimed sequence as part of any other (e.g. larger) protease substrate, therefore claim 20 is not suggested by the prior art.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Marjorie A. Moran whose telephone number is (703) 305-2363. The examiner can normally be reached on Monday to Friday, 7:30 am to 4 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Woodward can be reached on (703) 308-4028. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 872-9306 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 305-3524.

MARJORIE MORAN
PATENT EXAMINER

February 6, 2003